play a significant role in both non-polar and polar binding, which is comparable to water reorganization energy. For both solutes, the entropy of water reorganization is predicted to favor binding in agreement with the classical view of the ''hydrophobic effect'', which is countered by ligand entropy. Depending on the specifics of the binding pocket, both energy-entropy compensation and reinforcement mechanisms are observed. Notable is the ability to visualize the spatial distribution of the thermodynamic contributions to binding at atomic resolution, opening up exciting avenues for mechanistic investigations of protein-ligand binding.

1081-Pos Board B32

Markovian Milestoning for Computing Entry, Exit, and Internal Diffusion Rates of Ligands in Proteins

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Measuring diffusion rates of ligands plays a key role in understanding the kinetic processes inside proteins. For example, although many molecular simulation studies have reported free energy barriers to infer rates for CO diffusion in myoglobin (Mb), they typically do not include direct calculation of diffusion rates because of the long simulation times needed to infer these rates with statistical accuracy. We show in this talk how to apply Markovian milestoning along minimum free-energy pathways to calculate diffusion rates of both CO inside Mb and O2 inside monomeric sarcosine oxidase (MSOX). In Markovian milestoning, one partitions a suitable reaction coordinate space into regions and performs restrained molecular dynamics in each region to accumulate kinetic statistics that, when assembled across regions, provides an estimate of the mean first-passage time between states. We show here that the milestones can be chosen from a Voronoi tessellation defined by discrete centers taken from minimum free-energy pathways computed using the single-sweep reconstruction method. In the case of CO/Mb, we find that, although simulations predict the existence of many potential portals that connect the solvent phase with the distal pocket (DP, the site of CO attachment to the heme iron), the so-called ''histidine gate'' pathway is kinetically dominant for both CO entry and exit by approximately a factor of ten, in qualitative agreement with existing experimental data. Our calculations also semi-quantitatively agree with experiment, predicting a 60-ns mean exit time for CO from the DP and a mean entry time of 50 microsec under CO-saturating conditions. The Markovian milestoning approach seems from these results to be a promising way to estimate biomolecule-related transition rates, and ongoing work involves using it to time conformational changes.

1082-Pos Board B33

Improving Small Molecule Docking for Bcl-xL via Accelerated Molecular Dynamics with Cosolvent

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The anti-apoptotic factor Bcl-xL operates by binding and sequestering a range of pro-apoptotic factors that otherwise promote programmed cell death. Overexpression of Bcl-xL and its anti-apoptotic relatives is common in many human cancers. Consequently, extensive efforts are being directed to developing inhibitors of Bcl-xL protein-protein interactions. Here we describe the development of an improved workflow for utilizing experimental and simulated conformations in structure-based inhibitor design strategies. By employing computational docking of known small molecule inhibitors we demonstrate that certain methods of enhanced sampling molecular dynamics (MD) are more amenable to productive docking studies. In particular, structural ensembles derived from both accelerated MD (aMD) and MD in the presence of an organic cosolvent generally scored better than those from equivalent conventional MD. Furthermore, combined cosolvent aMD simulations yielded better average and minimum docking scores for known binders than an ensemble of 80 high-resolution experimental structures. A detailed analysis of the docked conformations indicates that the improved scores resulted from an increased flexibility of the helices flanking the main Bcl-xL binding groove. This enhanced conformational sampling allowed cosolvent to penetrate more deeply into the binding pocket and stabilize conformations not evident in conventional simulations. We believe this approach could potentially be used to identify protein-protein interaction inhibitors for other systems, and would be particularly beneficial against those targets for which there is less accumulated structural data.

1083-Pos Board B34

Free Energy Landscape of the Michaelis Complex of Lactate Dehydrogenase: A Network Analysis of Atomistic Simulations

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It has long been recognized that the structure of a protein is a hierarchy of conformations interconverting on multiple time scales. However, the conformational heterogeneity is rarely considered in the context of enzymatic catalysis in which the reactant is usually represented by a single conformation of the enzyme/substrate complex. Lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of two forms of the cofactor nicotinamide adenine dinucleotide (NADH and NAD+). Recent experimental results suggest that multiple substates exist within the Michaelis complex of LDH, and they are catalytic competent at different reaction rates. In this study, millisecond-scale all-atom molecular dynamics simulations were performed on LDH to explore the free energy landscape of the Michaelis complex, and network analysis was used to characterize the distribution of the conformations. Our results provide a detailed view of the kinetic network the Michaelis complex and the structures of the substates at atomistic scale. It also shed some light on understanding the complete picture of the catalytic mechanism of LDH.

1084-Pos Board B35

Dissecting Ligand Binding Sites : A Layer at a Time

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Biological phenomena at a molecular level are a consequence of complex interplay of interactions between ligands and their associated protein targets. Such interactions are localized to defined protein regions termed as binding sites (residues within ~4.5Å from ligand). Numerous cases exist wherein proteins of diverse folds bind to similar ligands. We undertake a study with an objective to quantify similarities in binding sites, that utilize the structural information of a binding site at atomistic detail, in order to examine extension of such similarities to additional regions surrounding the binding site. We find that 68% and 22% of high scoring comparisons among single-domain and multi-domain proteins respectively, exhibit similarity at the binding site that extrapolates to regions beyond it (an additional 4.5Å from binding site). Such similarities in binding sites as well as their extension to adjoining regions are analysed along with residue-wise correspondences and pairwise structural alignment of binding sites. Sequence identity and ligand topology are found to be independent of the extent of binding site similarity. Statistical validation of these findings re-iterates their relevance by means of p-value calculation as well as decoy site generation. Occurrences of similarity beyond the binding site are also further investigated using Protein Side-chain Networks (PScN). Such networks are created wherein each node represents amino acid residues, and an edge is constructed based on interaction strength between side chain of non-covalently bonded residues. PScNs lead to the identification of densely connected modules i.e cliques and communities in regions of binding site and surrounding areas. Thus, a combinatorial approach capturing atomistic details of binding site architecture and global perspectives of ligand-binding by PScNs could aid in providing valuable insights to current understanding of molecular recognition events.

1085-Pos Board B36

Encounter and Binding of Camp at the Binding Domain of Mlok1

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Ligand-protein binding processes play an essential role in biological systems, be it signalling immune response or enzymatic activity. An experimentally well-studied system is the binding of cyclic adenosine monophosphate (cAMP) at the cyclic nucleotide binding domain (CNBD) of the bacterial potassium channel MloK1. The channel's conductivity is modulated by cAMP binding and is prototypical for cyclic nucleotide gated ion channels.
Two models were postulated to describe the binding process: The first is a two-step model consisting of bulk-surface diffusion and surfing-binding site rolling. The second consists of a diffusion process into a binding funnel combined with stochastic barrier crossing.
Here we present a comprehensive molecular dynamics study of the primary cAMP binding events, i.e. the ligands path to the binding site in the CNBD,